REMARKS

Claims 23-27 have been rejected under 35 U.S.C. § 112, first paragraph; § 112, second paragraph; or § 103; and the Examiner has objected to claim 23. Claim 28 has been withdrawn by the Examiner as directed to non-elected subject matter. Applicants expressly reserve the right to file a divisional application directed to the non-elected subject matter. In view of the remarks below, Applicants respectfully request reconsideration and withdrawal of the asserted objections and rejections.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 23-27 have been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner contends that "neither the specification nor originally presented claims provides support for a process for preparing an agglutination reagent for detecting typhoid comprising preparing a polyclonal-monospecific antibody."[1] Applicants have deleted the recitation of "polyclonal-monospecific" from claim 23. Accordingly, withdrawal of this rejection is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

Claim 23 stands rejected under 35 U.S.C. § 112, second paragraph, as being indefinite because the limitation "said polyclonal-monospecific antibody specific to *Salmonella typhi*" lacks antecedent basis. Part (a) of claim 23 recites "preparing an antibody specific to a Flagellin gene of *Salmonella* typhi." Part (c) of claim 23 has been amended as follows "said ~~polyclonal-monospecific~~ antibody specific to said Flagellin gene of *Salmonella* typhi." Since part (a) provides antecedent basis for "said antibody specific to said Flagellin gene of *Salmonella* typhi," and part (c) has been amended to replace "said polyclonal-monospecific antibody ..." with "said antibody ...," Applicants respectfully request that this limitation be withdrawn.

[1] Office Action at page 3.

Rejection under 35 U.S.C. § 103

Claims 24-25 have been rejected under 35 U.S.C. § 103(a) as being obvious over Nilsson[2] in view of Sukosol[3]. Claims 23, 24, 26 and 27 stand rejected under 35 U.S.C. §103(a) as being obvious over Nilsson and Sukosol in view of Salzman[4] *et al.* (WO 01/040280) ("Salzman") and Fruitstone[5].

A. Recited Invention

The present invention is an agglutination test using latex particles coated with gamma-globulin fraction of serum prepared against S. typhi specific flagellin gene product. Thus, the results can be observed with the naked-eye, and without the aide of any instrument. Consequently, the recited invention can be used in the field.

To this end, claim 23 recites a process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising preparing antibody specific to a Flagellin gene of *Salmonella typhi*, preparing latex particles suspension, and coating of the latex particles with the antibody. The antibody is prepared by raising a hyper immune sera against a purified protein encoded by a Flagellin gene specific to *Salmonella typhi*.

The latex particle suspension is prepared in part by mixing 1% carboxylated latex particles and 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1, washing twice with 20 mM MES buffer of pH 5.5 thereby forming a washed latex particle. 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 is added to the washed latex particle.

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[2] Nilsson *et al.* "Microparticles for selective protein determination in capillary electrophoresis," ELECTROPHORESIS, (2001) 22: 2384-2390 ("Nilsson").

[3] Sukosol *et al.*, "Fusion protein of Solmonella typhi flagellin as antigen for diagnosis of typhoid fever," ASIAN PACIFIC J. OF ALLERGY AND IMMUN., (1994) 12:21-25 ("Sukosol").

[4] WO 01/040280 to Salzman *et al.* ("Salzman").

[5] U.S. Patent No. 4,379,847 to Fruistone *et al.* ("Fruitstone").

The washed latex particle is coated with essentially only the antibody fraction. The reaction is stopped with 1M glycine (pH 11.0), and then the antibody coated latex particle is washed with a buffer comprising 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05 % sodium azida.

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. The reagent comprises a carboxylated latex particle coated with an antibody specific to a Flagellin gene of *Salmonella typhi*. The particles are stored in a storage buffer. Claims 25-27 depend from claim 24 and further define the particle, storage buffer and antibody.

B. Cited References

Nilsson is directed to a system for detecting protein using two different monoclonal antibodies for human chorionic gonadotropin ("hCG").[6] There are two different monoclonal antibodies covalently bound to the latex particles.[7] Once the latex particles are created, Nilsson teaches washing the particles with Tris-BSA and blocking the carboxyl group with Tris-HCL.[8] When a test reagent is mixed with hCG containing sample, an immune complex is formed.[9] The complex is separated from the latex particles using capillary electrophoresis and detected by UV-Vis detection system.[10] Since Nilsson discusses using an instrument to detect the hCG, the system is confined to the laboratory, and cannot be used in the field. A capillary electrophoresis is different from an agglutination system.

Nilsson does not disclose several of the limitations recited in claim 23. Namely, Nilsson does not disclose: an antibody specific to *Salmonella typhi* Flagellin gene product because Nilsson is directed to hCG, washing the polyclonal monospecific coated latex particle with MES because Nilsson teaches washing with Tris-BSA, and blocking the carboxyl group

[6] Nilsson at abstract.

[7] Nilsson at abstract.

[8] Nilsson at page 2385.

[9] Nilsson at abstract.

[10] Nilsson at abstract.

with Tris-HCl, or an agglutination reagent because Nilsson teaches capillary electrophoresis. Nilsson also fails to disclose several limitations recited in claims 24-27. Namely, it fails to disclose: the recited antibody, an agglutination reagent, and the storage buffer.

Sukosol used a 900 base pair gene sequence specific to the *Salmonella typhi* flagellin gene to make a fusion protein with a GST tag in a vector for detection of the antibody (IgM) in serum samples of individuals suspected to have typhoid or related infection.[11] Sukosol does not disclose using the *Salmonella typhi* flagellin gene product for making an antibody, binding the antibody to a latex particle or using the antibody in an agglutination reagent.

Salzman generally is directed to a polypeptide derived from flagellin polypeptides used to generated an immune response to gram-negative bacteria.[12] Salzman used a portion of *Salmonella muenchen* specific flagellin gene product to prepare an antibody.[13] The gene product is comprised of less than 160 amino acids, which also match the flagellin amino acid sequence of other gram negative bacteria. Salzman used GST as a tag with the gene sequence to make the fusion protein.[14] Antibodies raised against this protein will not only react with clinical sample of *Salmonella muenchen* and other gram-negative bacteria, but will also react with parasitic infections caused by *Schistosoma japonicum.* Therefore, Salzman does not teach a polyclonal antibody, nor a monospecific antibody because the antibody generated according to Salzman's disclosure will not be specific to *Salmonella muechen.* Salzman also does not disclose (1) the recited antibody raised against Salzman's protein will react with *Salmonella muenchen,* other gram-negative bacteria and parasites such as *Schistosoma japonicum*, (2) using a latex particle, or (3) the recited reagents.

Fruitstone is directed to a suspending medium for use in immunological reactions, specifically immunohematologic reactions.[15] Although Fruitstone mentions in passing that its

[11] Sukosol at page 23, column 3.

[12] Salzman at abstract.

[13] Salzman at pages 11-21.

[14] Salzman at page 31.

[15] Fruitstone at col. 1, lines 10-14.

solution can be used in other immunologic reactions such latex particle agglutination tests,[16] it does not provide any experiments or other details for the use of its suspending medium in such reactions.

C. Argument

When making a rejection under 35 U.S.C. § 103, the Examiner has the burden of establishing a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, the prior art must be evaluated based on what it, as a whole, teaches to one of ordinary skill in the art. To establish this, each and every claimed element must be taught or made obvious by the applied references. Additionally, there must be some reason to combine the references in a manner that results in the recited invention.

Here, there is no reason to combine the references to result in a latex particle coated with the recited antibody. Furthermore, the references do not teach or suggest the recited blocking and washing steps, or the storage buffer.

Point I. There is no reason to eliminate Nilsson's second antibody

There is no reason why one would reasonably expect Nilsson's invention to work if the second antibody is removed from the latex particle. Assuming that one was motivated to combine the references, one would create a monoclonal antibody raised against Sukosol's 900 base pair gene sequence specific to *Salmonella typhi*. This antibody would be reacted with Nilsson's latex particle. This latex particle also includes a second antibody.

In contrast, claim 23 recites that the latex particle is coated with an antibody specific to *Salmonella typhi*, not two different antibodies. Likewise, claims 24-27 recite that the latex particle is coated with an antibody, not two different antibodies. Since there is no reason for one to have removed the second antibody from Nilsson's latex particle, the invention is not obvious over the cited references.

[16] Fruitstone at col. 4, lines 31-33.

A. Response to Office Action dated July 27, 2010

On page 12, the Examiner contends that "the Nilsson et al. reference clearly teach one type of antibody on each latex particle (see the description of Figure 1). Therefore, the general scheme taught by Nilsson et al. meets the limitations of the claim where there is one type of antibody bound to the particle. Furthermore, [claim 23] recite[s] 'comprising.'"[17]

Claim 23 has been amended to recite that the "latex particle is coated according to a method consisting essentially of (i) reacting said antibody specific to the Flagellin gene of *Salmonella* typhi with said washed latex particle thereby forming an antibody specific to the Flagellin gene of Salmonella typhi coated latex particle." Thus, as amended, claim 23 recites that the latex particle is prepared by reacting the particle with the antibody, and not additional antibodies. While Figure 1 in Nilsson teaches a first antibody and a second antibody bound to a first and second particle, it also teaches reacting both antibodies with both particles. Therefore, it does not teach a method consisting essentially of reacting a latex particle with one antibody.

Point II. There is no reason to substitute Nilsson's blocking and washing steps with the recited ones.

Additionally, the cited references do not teach the recited blocking and washing steps, and there is no reason to substitute Nilsson's washing and blocking steps with the recited washing step. Nilsson teaches blocking the residual activated carboxyl groups with 0.1 M Trus-HCl, pH 8.0 containing 0.2% BSA, and washing the antibody coated latex particles with Tris-BSA. However, claim 23 recites that the reaction between the latex particle coated and the polyclonal-monospecific antibody is stopped with 1M glycine (pH 11.0), and the polyclonal-monospecific antibody coated latex particle is washed with 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide.

There is no reason for one to substitute Nilsson's blocking and washing steps for the ones recited in claim 23. Accordingly, the recited invention is patentable over the cited references.

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[17] Office Action at page 12.

A. Response to Office Action dated July 27, 2010

Nilsson teaches using BSA to activate the particle and to stop the reaction. In contrast, claim 23 recites that the reaction is stopped with 1M glycine (pH 11.0), and that the coated latex particles are washed with a washing buffer comprised of 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide.

Additionally, the concentrations of reagents, pH and temperature play a critical role in *in vitro* immunological tests (see J. EXP. MED. (1924) vol. 39(2): 265; also see J. Exp. Med. (1926) vol. 44(5): 667; see also J. INFECT. DIS. (1933) vol. 53(1); see also J. IMMUNOL. (1948) vol. 58: 229; see also J. IMMUNOL. (1973) col. 111: 478; see also ESSENTIALS OF IMMUNOL. AND SEROL. (2002): 204-205).

Point III. There is no teaching or reason to use the recited storage buffer.

Neither Nilsson nor Sukosol teaches the storage buffer recited in claim 24 and further defined in claim 26. This deficiency is not overcome by Fruitstone because there is no reason to expect that Fruitstone can be applied to Nilsson's method.

A. Responses to Office Action dated July 27, 2010

Fruitstone discloses a suspending medium for immunohematologic reactions like antibody screening, antibody identification, crossmatches, antiglobulin testing, blood grouping, etc.[18] While, in passing, it mentions that "the solution may also be used in other types of immunologic reactions, such as latex particle agglutination tests,"[19] it does not provide sufficient evidence to enable one of ordinary skill to reasonably believe that this would be expected to work. It fails to provide any experiment details with respect to detection of viruses or bacteria. This information is necessary to provide one a reasonably expectation that the medium would not induce false positives, or, more specifically, the likelihood of false positives in latex particle agglutination tests.

[18] Fruitstone at col. 4, lines 26-35.

[19] Fruitstone at col. 4, lines 31-35.

Point IV. The secondary evidence rebuts any *prima facie* case of obviousness.

Notwithstanding the reasons set-forth above, even assuming that a *prima facie* case of obviousness has been established, the secondary evidence of long-felt but unresolved need, failure of others and commercial success rebuts the rejection. Typhoid is one of the most prevalent diseases afflicting countries in tropical regions of the world. It is also a very difficult disease to diagnose. The gold standard for diagnosing Typhoid is by taking a culture. However, this diagnostic test is very time consuming. Other methods include the Widal test, Widal slide agglutination Test, Radioimmunoassay, ELISA based antigen detection methods and commercially available Typhidot™. These methods all have several shortcomings, as listed in Table 1.

Table 1: Shortcomings of Typhoid Tests

Test	Shortcoming
Widal Test	Takes 18-25 hours after 6-7 days of enteric fever[20]
Widal Slide Agglutination Test	Take 1-3 min. after 6-7 days of enteric fever[21]
Culture Test	Takes 3-14 days after first day of fever[22]
ELISA based test	Takes 8 hours after 6-7 days of enteric fever
Radioimmunoassay	Very complicated and involve the use of radioactive material, which is not available in many countries where Typhiod is a health concern.[23]

[20] Specification at page 2, lines 5-6.

[21] Specification at page 2, lines 5-6.

[22] Specification at page 2, line 20 to page 3, line 16

[23] Specification at page 3, line 17 to page 4, line 3.

In contrast to these shortcomings, the claimed invention provides a process for the preparation of an agglutination reagent that can be used to detect typhoid early and rapidly with 100% specificity. The recited invention takes approximately 1-2 minutes to develop a color positive sample.[24] Since the invention relies on seeing a color, an untrained person can read the results. Thus, the invention is fast and easy to use in comparison to the prior art.

Furthermore, the invention can be used to detect typhoid at the onset of infection. The prior art is not capable of doing this.

Additionally, the invention is highly specific and sensitive for detecting typhoid. In contrast, the Widal test cross-reacts with other febrile organisms and other organisms within the Enterobacteriaceae family.[25] Also, it provides false positives when administered to a person who has been vaccinated, or when the appropriate baseline level of typhoid for a region is not known (typhoid, being an endemic, causes background level of antibody in endemic areas).[26] The culturing technique is also a low sensitivity test for typhoid (40 – 60 %) because there are very few organisms in circulation (as low as 1 / ml), which leads to false negatives.[27]

The agglutination reagent is stable for more than nine months at 4°C.

Finally, a leading Indian company has already taken interest in this invention.

Thus, there is a long-felt, but unresolved need for a fast and easy typhoid diagnostic test that can detect typhoid in its early stages. The prior art only teaches tests that are either time consuming (e.g. culture tests), or can only detect typhoid 6-7 days after enteric fever has been observed. Others who have tried to address this problem have failed by either developing a test that is time consuming, or can only detect typhoid 6-7 days after enteric fever.

[24] Specification at page 7, lines 17-19.

[25] Specification at page 1, lines 24-26.

[26] Specification at page 2, lines 1-4.

[27] Specification at page 3, lines 8-10.

In view of this secondary evidence, Applicants have rebutted any *prima facie* case of obviousness set-forth by the Examiner. In the absence of evidence to the contrary, the Applicants respectfully request that this rejection be reconsidered and withdrawn.

Point V (New). There is no reason to use an agglutination test.

Claims 23 and 24 are directed to agglutination tests, whereas Nilsson is directed to a capillary electrophoresis detection system. In Nilsson's system, when the antibody-coated latex particle reacts with the protein, the complex is separated from single latex particles using capillary eletrophoresis and detected by ultra violet diode array detection or laser-induced fluorescence imaging detection.[28]

Sukosol does not overcome this deficiency because it is not directed to an agglutination system. Sukosol is solely directed to the existence of a 52kDa antigen of *Salmonella typhi* detected by monoclonal antibodies via Western blot and ELISA assays. These methods do not permit one to detect *Salmonella typhi* with the naked eye.

Objection to Claim 23

The Examiner has objected to claim 23 because it contains a typographic error in the recitation of "*typhi*coated." Applicants have corrected this typographic error. Accordingly, withdrawal of this rejection is respectfully requested.

[28] Nilsson at page 2384, col. 1.

Conclusion

Due to the differences discussed above, a combination of the cited references do not result in the recited invention. For these reasons, Applicants respectfully request reconsideration and withdrawal of the objections and rejections, allowance of pending claims 23-27, and rejoinder of claim 28.

Respectfully submitted,

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